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RHYTHMS IN LARVAL RELEASE BY AN ESTUARINE CRAB (*RHITHROPANOPEUS HARRISII*)

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Abstract

Ovigerous females of the crab *Rhithropanopeus harrisii* were collected subtidally, and their rhythms in larval release monitored under constant conditions in the laboratory. Larvae from a single crab are generally released as a burst lasting less than 15 minutes. Larval release by crabs from an estuary lacking regular tides mainly occurs in the 2-h interval after sunset and is not related to coastal tides, which suggests a circadian rhythm. This rhythm can be entrained on an altered light-dark cycle. Larval release by crabs from an estuary with semi-diurnal tides begins at high tides and continues for 2 hours, suggesting a circatidal rhythm. Significantly more releases occur during the night. Crabs from the estuary without regular tides change from a circadian to a circatidal rhythm after being in the estuary with semi-diurnal tides. Alternatively, crabs from the estuary with semi-diurnal tides change to a circadian rhythm when exposed to a light-dark cycle and non-tidal conditions in the laboratory. Thus *R. harrisii* has both circadian and circatidal rhythms in larval release with the expressed rhythm dependent upon prior environmental conditions. Nighttime release may reduce predation, while release at high tide may minimize larval exposure to stressful, low salinity water.

Introduction

Rhythms in reproductive activity and larval release are common among crustaceans. Timing may be related to lunar phase, time of day, and/or phase of the tide. Semilunar cycles are known for semi-terrestrial crabs (Gifford, 1962; Warner, 1967; Henning, 1975; Klassen, 1975; Saigusa and Hidaka, 1978; Seiple, 1979; Saigusa, 1981), intertidal fiddler crabs (von Hagen, 1970; Zucker, 1976, 1978; Christy, 1978; Wheeler, 1978), and subtidal stomatopods (Reaka, 1976). For the lobsters *Nephrops norvegicus* (Moller and Branford, 1979), *Homarus gammarus* (Ennis, 1973; Branford, 1978), and *H. americanus* (Ennis, 1975), larval release in the laboratory occurs shortly after dusk on a series of consecutive nights; no lunar or semilunar rhythm has been reported. *H. americanus* occasionally releases larvae during the day (Ennis, 1975).

Detailed laboratory studies of estuarine intertidal fiddler crabs indicate that females release their larvae within several hours after the time of the nocturnal high tide (DeCoursey, 1979; Bergin, 1981). As implied by DeCoursey (1979), precisely timed larval release may not be restricted to intertidal fiddler crabs but could also extend to other estuarine species.

This study was undertaken to examine larval release by the estuarine crab *Rhithropanopeus harrisii* which occurs from the very low intertidal zone into subtidal

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areas (Williams, 1965). Crabs from an estuary having pronounced semi-diurnal tides were compared to crabs from an estuary lacking regular tides. Experiments were designed to determine the presence of biological rhythms in larval release, the relationship of release time to environmental cycles, and the ability of the crab to change its rhythm under different environmental conditions.

Materials and Methods

Ovigerous female *Rhithropanopeus harrisii* (Gould) were collected from two coastal estuaries in North Carolina, the Neuse River (estuary A) and the Newport River (estuary B). Tides in estuary A are aperiodic (Roelofs and Bumpus, 1953). Physical factors such as salinity, water depth, and wave turbulence which usually vary with the tides, vary instead with wind direction and rain. In contrast, estuary B has regular semi-diurnal tides and periodic variation in tide-related hydrography (Cronin, 1982).

Crabs were obtained in wire mesh traps. In estuary A traps were placed at a depth of about 1 m on a gradually sloping bottom. Traps in estuary B were placed in an area having a relatively uniform depth of 3–4 m at high tide.

Ovigerous crabs were collected during the day, separated in the laboratory according to embryo development (based on yolk consumption and eye development), and placed in 20-cm diameter finger bowls containing water of the same salinity as at the collection site. The proper salinity was obtained by diluting sea water (filtered to remove particles larger than 5 μm) with distilled water. Crabs were either placed under constant conditions of temperature, salinity, and light, or entrained to a new LD cycle in an environmental chamber (Sherer Gillett Co., Model CEI 4-4). None were fed.

Larval release usually occurs during a specific interval in the LD or tidal cycle. The time was determined by intensively monitoring larval release over a designated 5-h sampling interval within the LD or tidal cycle. During this 5-h period, crabs were transferred every 15 min to a new 7.9-cm diameter finger bowl. At the end of the sampling period, the crabs were placed in 10.4-cm diameter finger bowls, and if eggs remained, the procedure was repeated at the next monitoring time, either 7.4 or 19 h later (see below).

The number of larvae released within each 15-min interval and between sampling intervals was recorded. Most larvae are released within 15 min, though a few commonly appeared in the intervals immediately preceding and following the peak. The mean time was calculated by multiplying the number of larvae released per 15 min by that interval, taking the sum of these products over all intervals, and dividing this sum by the total number of larvae. In this way a single 15-min interval was designated as the time of larval release. If a crab released the majority of its larvae during the period between the 5-h sampling periods, release was designated as occurring at "other times." About 12% of the crabs released bursts of larvae during two consecutive sampling periods. Using the above procedure a mean time was calculated for each release.

A chi-square test for goodness of fit was used to determine whether the number of releases during the intensive sampling time differed from an expected uniform rate throughout the solar day or over an entire tidal cycle. For these tests, the solar day included a sampling time and the preceding 19 h, while a 12.4-h tidal cycle encompassed the sampling time and the preceding 7.4 h. For crabs from estuary B, a chi-square test was used to determine any preference for releasing at day or nighttime high tides. A Kolmogorov-Smirnov goodness of fit test was used to determine

if releasing was nonuniform throughout the intensive sampling time. Finally, linear regression analysis was used to estimate the period length of the rhythms by the population. For crabs, which release a burst of larvae during two consecutive sampling intervals, only the time of the first burst was used in this analysis. In this way each crab only contributed one time to the data. Larval release was monitored in five situations. The specific procedures for each situation are described in the next section.

Results

Estuary A: crabs from natural conditions

In preliminary experiments larval release by crabs from estuary A was monitored at 2-h intervals under constant laboratory conditions for 3 days. Releases began just after sunset and continued for several hours. Releasing could be related to time of day or perhaps to tides, even though tides are considered aperiodic at the collection site.

To distinguish between these possibilities crabs were collected at weekly intervals for one month (May 16 to June 13, 1981). The tidal phase at dusk on the nearby coast alternated weekly between spring high tides and neap low tides. After collection and embryo staging, all crabs were maintained under room lights until the time of normal sunset when they were placed in constant low level light (photographic safelight containing a 15-W bulb and fitted with a Kodak OA filter; wavelength maximum = 573 nm, half band pass = 37 nm, intensity = 1.2×10^{-2} W/m^2), and temperature (28 ± 1°C). A crab remained under constant conditions until it released its larvae or until 6 nights had elapsed and the experiment was terminated. Beginning 1 h before the time of the first sunset, all crabs with advanced embryos were transferred through the series of finger bowls. Other crabs were tested as their embryos matured.

At both collection times, each crab had embryos at one stage of development. However within the collected crabs, embryo development was not uniform, as all stages were observed. The number of crabs that released larvae in the laboratory within 6 nights of collection was similar at both collection times (high tide collection, n = 133; low tide collection, n = 110). These results suggest there is no lunar or semi-lunar cycle in larval release.

During spring high tides significantly more releases occurred during the sampling time on nights 1–5 (Fig. 1A; nights 1–4, $P < 0.005$; night 5, $P < 0.025$) than expected if releasing occurred uniformly throughout the solar day. Furthermore, releasing was not uniformly distributed within the 5-h sampling intervals on nights 1–4 and 6 (nights 1 and 6, $P < 0.01$; night 3, $P < 0.02$; nights 2 and 4, $P < 0.05$). Therefore larval release by the population occurred during a relatively short time within the 4-h interval after sunset.

Similarly, when low tides occurred during the evening the total number of releases during the intensive sampling time was greater than expected (Fig. 1B; nights 1–6, $P < 0.005$). Furthermore, releasing within the sampling time was nonuniform on nights 1, 3, 4, 5, and 6 (night 4, $P < 0.01$; night 6, $P < 0.02$; nights 1, 3, and 5, $P < 0.05$). Again larvae were released mainly within several hours after sunset.

There was no significant difference in the distribution of release times on specific nights during evening high and low tides (Mann-Whitney U test). (*i.e.*, comparison of nights 1, nights 2, *etc.*). To further compare the two situations, a regression was determined for the relationship of release time and night in constant conditions. Night 1 was excluded because release times may be influenced by initial adjustments

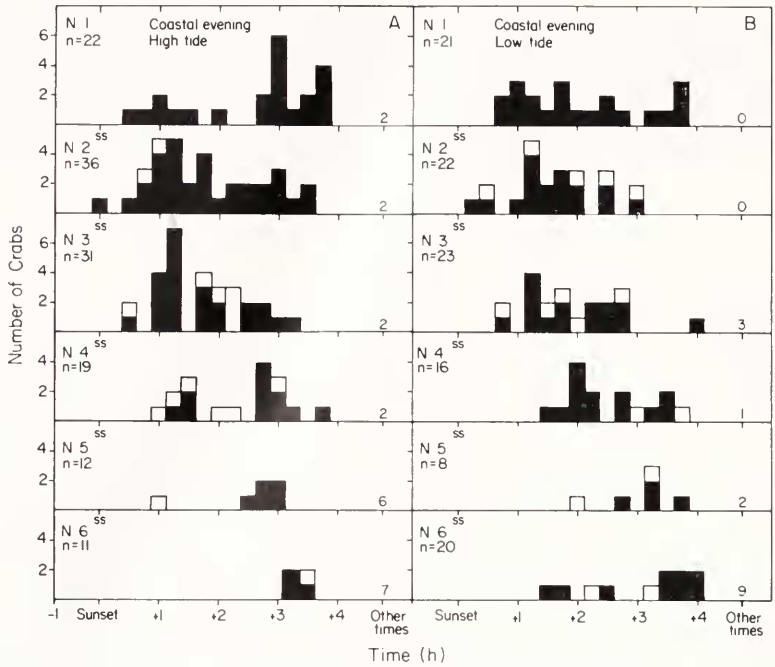

FIGURE 1. Number of crabs releasing larvae (ordinate) at times (abscissa) relative to sunset in estuary A. Crabs were under constant conditions in the laboratory and releasing was monitored during a 5-h interval on successive nights (N) when spring high tides (A) or neap low tides (B) occurred during the evening on the nearest coast. On N1 in A high tide occurred at about the time of sunset while low tide occurred at this time in B. The sample size (n) on each night is shown and "other times" indicates the number of releases at times other than the sampling time. For example the releases shown on N2 for "other times" indicate those that occurred between the end of the first and beginning of the second sampling interval. The second release of a crab is indicated by an open histogram.

to laboratory conditions. The slopes of the regression lines for releasing at both evening high and low tides were significantly different from zero (t-test; $P < 0.01$). When the two regressions were compared by an analysis of covariance, neither the slopes nor the intercepts were significantly different (F-test). These findings suggest release time is not related to coastal tides, and the data in Figures 1 and 2 were therefore combined for the following analysis.

Larval release predominantly occurred within a specific time interval on consecutive days in constant conditions, which suggests the crabs have an endogenous rhythm. The period of the rhythm of individual crabs can be estimated from the time between consecutive larval releases. Nineteen percent of the crabs released on two consecutive nights. The mean time between releases was 24 h (SE = 15 min; n = 25), when rounded off to the nearest 15-min interval.

In addition the period length of the population rhythm can be estimated by a regression analysis of the relationship between release time and night after placement in constant conditions (Fig. 2). Night 1 was not included, and only releases during the sampling interval were considered. On nights 5 and 6 the number of crabs releasing at times other than the intensive sampling time increased. Nevertheless these nights were included because a significantly ($P < 0.005$) greater number of crabs released in the 5-h sampling interval than predicted if releasing was uniform over the solar day. The slope of the regression line (Fig. 2) is significantly different

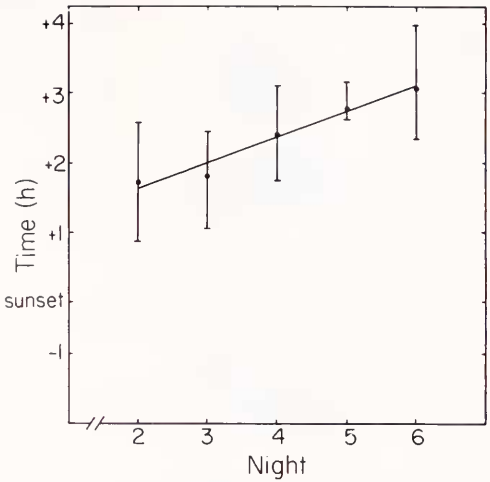

FIGURE 2. Regression of time of larval release (ordinate) for the combined data from Figures 1 and 2 on the consecutive nights in the laboratory (abscissa). The first release by all crabs within the intensive sampling interval was used for the analysis while the mean and standard deviation are shown on the figure.

from zero (t-test; $P < 0.001$), and predicts a time of 24 h 23 min between consecutive releases. Thus both the time between releases by a single crab and the regression analysis of the population indicate the free running period length is near 24 h. This implies the presence of a circadian rhythm in constant conditions in the laboratory and a daily rhythm in nature.

Estuary B: crabs from natural conditions

Larval release by these crabs occurs near the time of high tide (Cronin and Forward, 1982). To determine the precise relationship between release time and tide, crabs were collected at weekly intervals several hours before daytime high tide during July and August, 1980 and 1981. Since larval release was monitored in crabs collected in the same area at the same time of the year, data from the two years were pooled. Crabs were collected at a depth where daylight probably is not visible (see below); thus crabs were returned to the laboratory in opaque bottles and then sorted according to state of embryo development. The only assured times the crabs experienced light were the short intervals during removal from the traps and when the embryos were staged. All crabs were then placed under constant conditions and larval release monitored as described previously. The first sampling time began just after staging of embryos. For this tide, crabs with the most advanced embryos were monitored for 4 h beginning about 1 h before high tide in the field. On the following 6 tides, crabs with mature embryos were monitored from 2 h before high tide in the field to 3 h later.

Larval release by crabs from estuary B is not uniform (tides 1–7; $P < 0.005$) over a complete tidal cycle (Fig. 3). Ninety-three percent of all releases occurred near high tide during the sampling time. Within this interval, releasing was distributed uniformly except for tides 1 and 2 ($P < 0.01$), when most larvae were released in the 2-h interval after high tide.

Releasing occurred near the times of high tides, which suggests the crabs have a biological rhythm. At the individual level, fifteen percent of the crabs (n = 25)

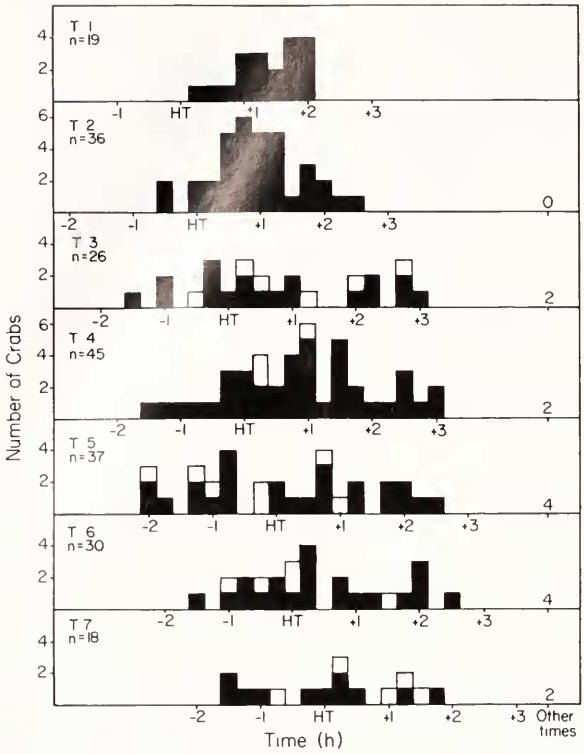

FIGURE 3. Number of crabs releasing larvae (ordinate) at times relative to high tide (HT) in estuary B. Larval release was monitored for a 5-h period around HT at the times of successive high tides (T) in the field (abscissa). The sample size (n) for each high tide is shown and "other times" indicates the number of releases at times other than the sampling interval. For example, the releases shown on T3 for "other times" are the number that occurred between the end of the second and beginning of the third sampling interval. The second release of a crab is indicated by an open histogram.

released larvae during successive high tides, while about 24 h elapsed between releases by one crab. The mean time between successive releases was 12 h 15 min (SE = 15 min) when rounded off to the nearest 15-min interval. The relationship between larval release by the population and tide (Fig. 4) is significant (t-test; $P < 0.001$). Furthermore, the slope of the regression line indicates the time between releases on consecutive tides is 12 h 12 min. Multiple releases by a single crab as well as the regression analysis of the population indicate that the free running period length is around 12 h 15 min. This implies the presence of a circatidal rhythm under constant conditions in the laboratory and a tidal rhythm in nature.

Although releases occurred on successive tides, it is possible that there is a day/night component in the rhythm. The average natural photoperiod throughout the experiments was 14 h light and 10 h dark. If releasing is independent of the light-dark cycle, then the predicted frequencies during the day and night sampling intervals would be 58 and 42%, respectively. The observed frequencies during the day and night are 48 and 52%. Releasing was not uniform during daytime and nighttime high tides (chi-square test, $P < 0.005$), as a significantly greater amount occurred near the time of nighttime high tides.

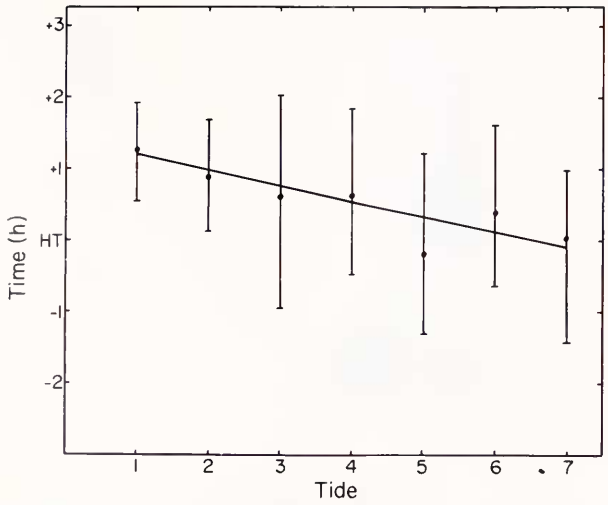

FIGURE 4. Regression of time (± SD) of larval release (ordinate) on the various tides (abscissa) for crabs from estuary B. The first release by all crabs within the intensive sampling interval was used for the analysis while the mean and standard deviation are shown on the figure.

Estuary A: entrainment to a LD cycle in the laboratory

Two methods were used to determine if the apparent circadian rhythm of crabs from estuary A could be entrained by a light-dark cycle. In the first method, crabs were collected during the day and those with embryos in early stages of development were held at 27.0°C under a 14:10 LD cycle in the environmental chamber. The light intensity during the day phase was about 2.0 W/m^2 (cool white fluorescent lamps). The length of the photoperiod was similar to that in the field, but the beginning of the dark phase occurred 6 h before sunset. The crabs were maintained under these conditions for 5 days because preliminary experiments showed this duration was sufficient to reset the timing of the rhythm. Crabs were then placed under the same constant conditions as described above for crabs from estuary A. The method for monitoring release was also similar except that the sampling time began 1 h before the end of the light phase. Crabs were monitored for 3 days.

The time of larval release shifted with an altered LD cycle in the laboratory. When the time of "lights off" (laboratory sunset) occurred 6 h before the normal sunset in the field, the time of releasing shifted similarly after 5 days of entrainment (Fig. 5). Clearly, releasing was not uniform over the day, since it only occurred during the sampling time. Within this interval, releasing was also not uniform on all 3 nights ($P < 0.05$). Larval release began at the end of the light phase and continued for about the next 1.5 h. A regression analysis of the population release times was not performed because crabs were monitored only over 3 days. Nevertheless, at the individual level 13% of the crabs (n = 10) released larvae on consecutive nights. The mean time between releases was 24 h 15 min (SE = 15 min) when rounded off to the nearest 15-min interval, which approximates the suggested free running period length of population from the field (Fig. 2).

The second method involved monitoring larval release by crabs that were maintained under summer conditions during the winter in a laboratory habitat. Crabs

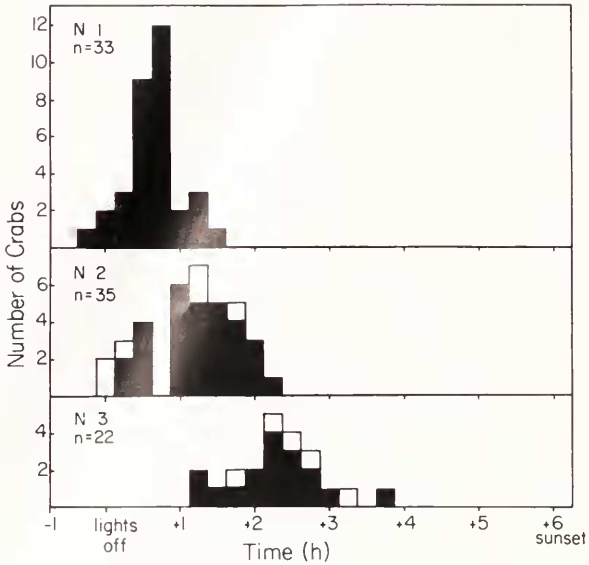

FIGURE 5. Number of crabs from estuary A releasing larvae (ordinate) at times (abscissa) relative to the end of the light phase. Crabs were placed on a 14:10 LD cycle in the laboratory for 5 days and then maintained under constant conditions. "Lights off" indicates the end of the light phase on the first night and "sunset" is the time of sunset in estuary A. Other symbols, as in Figure 1. No releases occurred at other times.

were held at 26.0°C in 9 ppt sea water, subjected to a summer photoperiod (15:9 LD cycle; cool white fluorescent lamps positioned over the tank; intensity = 7.5 W/m^2) and fed with Purina cat chow. Females became ovigerous in January and breeding continued through the spring. The method for monitoring releasing was identical to that for the previous crabs on an altered LD cycle. However during constant conditions the crabs were placed in the environmental cabinet (temperature, 26°C) having low intensity red light (6.5-W red incandescent lamp; wavelength output was greater than 600 nm; intensity about 0.3 W/m^2). Releasing was monitored for 3 days.

Winter crabs also exhibited a rhythm in larval release that was entrained to the altered LD cycle (Fig. 6A). Only 4% of the crabs did not release larvae in the sampling time which indicates releasing was nonuniform over the solar day ($P < 0.005$). On all 3 nights the release distribution was not uniform within the sampling interval ($P < 0.01$). Again releasing began at the end of the light phase and continued for about the next 1.5 h. Eleven percent of the crabs (n = 5) released larvae on consecutive nights, with a mean time between releases of 23 h 30 min (SE = 15 min) when rounded off to the nearest 15-min interval.

Estuary B: entrainment to a LD cycle in the laboratory

To determine whether crabs from estuary B could change from an apparent circatidal to a circadian rhythm, crabs with embryos which would hatch between 6 and 9 days after capture were collected shortly before high tide and placed in the environmental chamber under a 14:10 LD cycle (cool white fluorescent lamps plus a 60-W incandescent bulb; intensity = 9.0 W/m^2) at 27°C. In order to separate daily and tidal influences, the time of the LD cycle was adjusted so that the dark phase

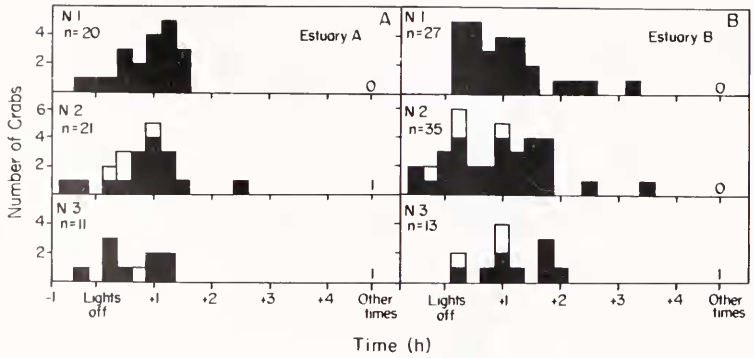

FIGURE 6. Larval release by crabs from estuary A (A) and estuary B (B) that reproduced during the winter. Crabs were maintained on a 15:9 LD cycle, and releasing was monitored under constant conditions. "Lights off" indicates the end of the light phase on the first night. Other symbols, as in Figure 1.

began at the predicted time of daytime low tide in the field 6 days after collection. After 6 or 7 days crabs were placed under constant conditions similar to those used for the tidal rhythm experiments with crabs from estuary B and larval release was monitored intensively for 5.5 h, beginning 1 h before the beginning of the dark phase. Preliminary experiments indicated that 6 days was the minimum time necessary for the crabs to change their rhythm. Releasing was monitored for only 3 days because the total time for embryonic development is about 10 days. Few crabs still had eggs at the end of the experiment.

Crabs released larvae during the 1.5-h interval after the end of the light phase (Fig. 7) rather than during the time following high tide in the field. Releasing was neither uniform during the solar day ($P < 0.05$) nor during the sampling interval for all nights ($P < 0.05$). Eight percent of the crabs (n = 5) released larvae on consecutive nights. The mean time between releases was 24 h (SE = 15 min).

To further establish that crabs from estuary B can develop a circadian rhythm, crabs were maintained over the winter in a habitat identical to that used for crabs from estuary A, and releasing was monitored using similar procedures. Larval release by winter crabs was also related to the LD cycle (Fig. 6B), as only 1% of the crabs did not release larvae during the intensive sampling interval (nonuniform releasing over the solar day, $P < 0.005$). Releases were nonuniform within the sampling interval on all nights ($P < 0.05$) with most releases occurring in the 1.5-h interval after the end of the light phase. Twelve percent of the crabs (n = 8) released larvae on consecutive nights. The mean time between releases was 23 h 30 min (SE = 15 min), which corresponds to the time observed for crabs from estuary A under similar conditions (Fig. 6A).

Estuary A: entrainment to natural tidal conditions

A final experiment determined whether crabs from estuary A could change from an apparent circadian to a circatidal rhythm in larval release. Since the environmental cycles which entrain the tidal rhythms are unknown, male and nonovigerous female crabs from estuary A were placed in plastic boxes containing mollusk shells in traps at the collection site in estuary B. Holes in each box permitted water flow through the box but prevented the crabs from escaping. Beginning 10 days after crabs were translocated to estuary B, ovigerous females were collected at weekly

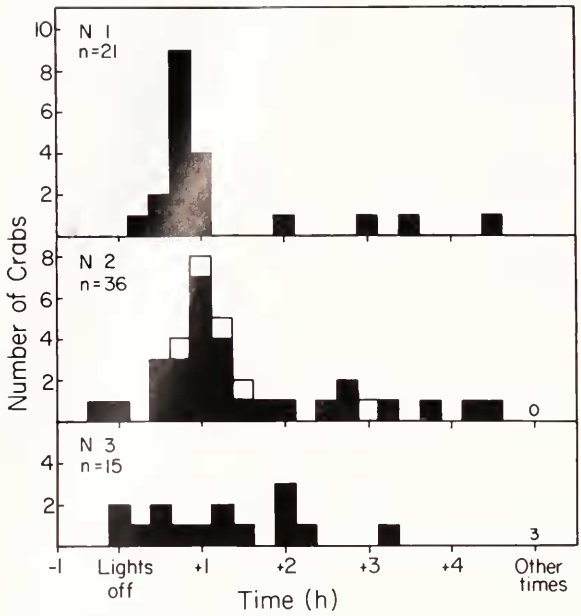

FIGURE 7. Number of releases (ordinate) at times (abscissa) relative to the end of the light phase for crabs from estuary B which were placed on a 14:10 LD cycle in the laboratory for 6 or 7 days and then maintained under constant conditions. "Lights off" indicates the end of the light phase on the first night. Other symbols, as in Figure 1.

intervals for a month. The crabs were collected several hours before daytime high tide, and larval release measured as described for crabs from estuary B (tidal experiments).

When crabs were transferred from estuary A to estuary B, they developed an apparent circatidal rhythm (Fig. 8). Eighty-four percent of all releases occurred during the 5-h sampling time around high tide (nonuniform releasing over the tidal cycle, $P < 0.005$), though releasing was uniform within this interval on all tides. There was no significant preference for day or night high tides. Three of the crabs released larvae during consecutive sampling times.

DISCUSSION

The crab *Rhithropanopeus harrisii* shows rhythms in larval release that are related to environmental cycles in the habitat where it lives. At the collection site in estuary A, crabs experience the natural light-dark cycle and a diel temperature cycle (unpublished observations). Tides in this area are aperiodic (Roelofs and Bumpus, 1953) even though tidal currents may occur in the estuary (Knowles, 1975). In the experiments, larval releases of crabs from this estuary were not related to tides but rather began at sunset and continued for about 2 h (Fig. 1). The observed time of larval release does not reflect the monitoring regime or handling, since the identical release pattern was observed in preliminary experiments when crabs were handled every 2 h for 3 days.

The relationship between releasing and the time of sunset suggests the presence of a circadian rhythm. A circadian rhythm is normally defined as an endogenous rhythm which persists for at least 5–10 cycles in a single individual under constant

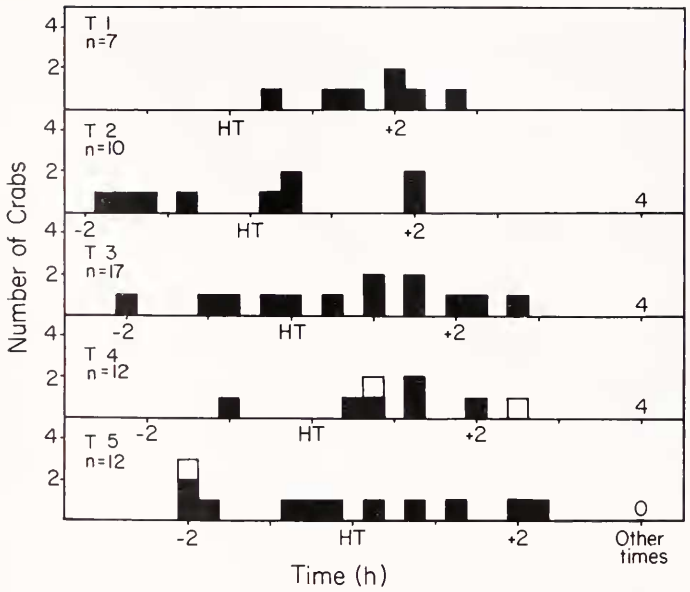

FIGURE 8. Larval release by crabs from estuary A that were transplanted to estuary B. Notations as in Figure 3.

conditions and which has a free running period close to but not exactly 24 h. Larval release occurs either as a single event in one crab or, at most, two events on consecutive nights. Thus the criteria of persistence of the rhythm in a single individual for 5–10 cycles cannot be fulfilled. Nevertheless, the rhythmic release of larvae by a population of crabs does persist under constant conditions in the laboratory for at least 6 diel cycles (Fig. 1). Also, the time between consecutive releases by a single crab and the regression analysis of population release times (Fig. 2) indicate free running period lengths of nearly 24 h. These considerations suggest that there is a circadian rhythm in larval release by individual crabs in constant conditions in the laboratory which is observed as rhythmic releases by the population within a specific time interval on successive days. Furthermore, these results indicate that the population has a daily rhythm in larval release in nature.

In contrast, crabs from an estuary with semi-diurnal tides had the greatest number of releases in the 2-h interval after high tide (Fig. 3). This pattern is not due to the sampling regime, since similar results were obtained in preliminary experiments when the crabs were sampled every 2 h for 6 days (Cronin and Forward, 1982). These results suggest the presence of a circatidal rhythm. The rhythmic release of larvae by the population persisted for seven tidal cycles under constant conditions and had a free running period of about 12 h 15 min (Fig. 4) as did successive releases from a single crab. In nature the population would have a tidal rhythm in larval release.

These crabs also had a significantly greater number of larval releases at night, although a considerable number of releases occurred during daytime high tides. The absence of a strong preference for day or night may be due to environmental conditions. The crabs were collected in traps at a depth of 3–4 m. Here they would be exposed to pronounced tidal changes in current flow, depth, and salinity (Cronin, 1982), but it is unlikely that they would sense a diel LD cycle. This prediction is

based upon the rapid attenuation of light in the estuary and assumes that the adults have the same spectral (Forward and Cronin, 1979) and intensity sensitivity (Cronin, 1979) as their larvae. Since the crabs can move from shallow to deep areas, we were probably sampling crabs which had and had not been exposed recently to the natural LD cycle. This may explain the weak preference for night in our results.

The crabs change both the time of larval release and the length of the free running period when exposed to different environmental cycles. Crabs from estuary A altered the time of releasing when entrained to a new LD cycle in the laboratory (Figs. 5 and 6A). Under a LD cycle and nontidal conditions in the laboratory, crabs from estuary B developed a circadian rhythm (Figs. 6B and 7). All crabs (estuaries A and B) that were entrained to a LD cycle in the laboratory, began larval release just after the end of the light phase and continued for about 2 hours. Releasing occurred at the same time for field-captured animals (Fig. 1), which suggests the LD cycle is the normal zeitgeber in the field.

A circatidal rhythm was induced in crabs from estuary A by placing them in estuary B (Fig. 8), but the zeitgeber remains unknown. The phase of the rhythm was similar to that for crabs living in estuary B. Interestingly, these crabs had a pronounced daily rhythm in their original habitat, yet lacked a day/night preference after exposure to conditions in estuary B. The transported crabs were placed in boxes at 3–4 m depth with no possibility of movement to shallower depths. It is unlikely the crabs experienced a LD cycle at this depth, which may explain the absence of a preference for larval release at night.

The crabs from both estuaries have the capability of showing both circatidal and circadian rhythms after exposure to different environmental conditions. Larval release by *R. harrisii* is not the only example of this flexibility. For example, this was also found in activity rhythms of the crabs *Carcinus maenas* (Naylor, 1958, 1960) and *Uca tangeri* (Altevogt, 1959) and in vertical migration patterns of *R. harrisii* larvae (Cronin and Forward, 1979). Of particular interest, however, is the time of release with respect to the tidal and diel LD cycles, since larval release could be most adaptive at a particular time of day or phase of the tide.

When not exposed to periodic tides, *R. harrisii* shows a daily rhythm with releasing occurring primarily in the 2-h interval after sunset. Larval release at night is commonly observed in laboratory studies of crustaceans such as lobsters (Pandian, 1970; Ennis, 1973, 1975; Branford, 1978; Moller and Branford, 1979), fiddler crabs (DeCoursey, 1979, 1981; Bergin, 1981), and the prawn *Macrobrachium idae* (Pandian and Katre, 1972). Nighttime release has been observed in field studies of fiddler crabs (Hyman, 1922; Christy, 1978; DeCoursey, 1981; Stancyk and Christy, 1981), pebble crabs (Knudsen, 1960), *Cardisoma guamhumi* (Gifford, 1962), *Aratus pisoni* (Warner, 1967), *Birgus latro* (Reese and Kinzie, 1968) and various *Sesarma* species (Saigusa, 1981). This suggests nocturnal larval release has a common functional advantage, which is probably avoidance of predators on larvae and adults which visually sight and actively pursue their prey (Ennis, 1975; Branford, 1978; DeCoursey, 1979; Bergin, 1981).

Larval release by *R. harrisii* from a tidal area occurs primarily in the 2-h interval after the time of high tides. In other detailed studies of crustaceans from an estuarine tidal area, fiddler crabs (DeCoursey, 1979, 1981; Bergin, 1981) and *Sesarma* sp. (Saigusa, 1981) had similar times of larval release. Since releases frequently occur near high tide, this again suggests a common functional advantage.

In estuaries, larvae encounter the problems of transport and of survival in a highly variable environment. By entering the water column near the time of high tide, subsequent horizontal movements are seaward as the tide recedes. This would tend to favor larval dispersal. Although this explanation is appropriate for fiddler

crabs (Wheeler, 1978; Christy, 1978; Bergin, 1981; Stancyk and Christy, 1981) and *Sesarma* sp. (Saigusa and Hidaka, 1978; Saigusa, 1981), it is unlikely that the time of release by *R. harrisii* aids dispersal, since field studies (Bousfield, 1955; Pinschmidt, 1963; Sandifer, 1973, 1975; Cronin, 1982) indicate that massive seaward transport of larvae does not occur, and that all larval stages are retained in the area of the adult population (Cronin, 1982).

A more reasonable hypothesis is that larval release near the time of high tide functions as adaptation to avoid stressful or even lethal salinity conditions. Estuarine benthic crabs are exposed to changes in salinity over a tidal cycle, with the upper value rarely exceeding 35 ppt. Salinity tolerances of larvae from estuarine crabs are usually sufficient to cope with the maximum salinity values they are likely to encounter. For example, successful larval development of *R. harrisii* occurs between 2.5 and 40 ppt (Costlow *et al.*, 1966). The real tolerance problem is low salinity water, which would be experienced at low tide. Since salinity is potentially highest and thereby least stressful around high tide, this would be an appropriate time for estuarine crabs to release their larvae.

Both von Hagen (1970) and Saigusa (1981) also consider the timing of larval release to be related to salinity tolerance. They both suggest that if larvae are released around the time of spring high tide, the subsequent ebb would transport them towards the ocean where they would encounter less stressful, high salinity water. Since *R. harrisii* larvae are not transported to the ocean, larval survival may depend upon exposure to the highest possible salinity at the time of release.

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